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Effect of Sodium Tetrathionate on the Activities of Some Enzymes in Kidney and Urine

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Summary: The activities of lactate dehydrogenase, glutamate dehydrogenase, aspartate aminotransferase, β-galactosidase, N-acetyl-β-*D*-glucosaminidase, leucine aminopeptidase, γ-glutamyltransferase and alkaline phosphatase in renal tissue and urine of rats treated with sodium tetrathionate were determined. A decrease of enzyme activities in renal tissue and an increase in urine were observed.

The largest decrease in the glutamate dehydrogenase of renal tissue amounted to 0.7 times the control value, and was correlated with an appropriate increase in the urine. Increases in urinary enzyme activity were especially marked for β-galactosidase and N-acetyl-β-*D*-glucosaminidase (3 and 6 times the control values, respectively). The increase in enzyme activities was not accompanied by a corresponding change in the urinary protein. Characterization of urinary lactate dehydrogenase and N-acetyl-β-*D*-glucosaminidase isoenzymes also indicates the renal origin of these enzymes.

The abnormally high enzyme activities of the urine correlated with the nature and degree of renal damage shown by electron microscopy.

Wirkung von Natriumtetrathionat auf die Aktivitäten einiger Enzyme in Niere und Harn

Zusammenfassung: Die Aktivitäten von Lactatdehydrogenase, Glutamatdehydrogenase, Aspartataminotransferase, β-Galaktosidase, N-Acetyl-β-*D*-glucosaminidase, Leucinaminopeptidase, γ-Glutamyltransferase und alkalischer Phosphatase in Nierengewebe und Harn mit Natriumtetrathionat behandelter Ratten wurden bestimmt. Es wurde ein Abfall der Enzymaktivitäten im Nierengewebe und eine Zunahme im Harn beobachtet.

Glutamatdehydrogenase im Nierengewebe zeigte mit einem Abfall bis auf einen Bruchteil von 0,7 des Kontrollwertes den stärksten Abfall; dieser war korreliert mit einem entsprechenden Anstieg der Ausscheidung im Harn. Ein Anstieg der Aktivität im Harn wurde besonders für β-Galaktosidase mit dem dreifachen und N-Acetyl-β-*D*-glucosaminidase mit dem sechsfachen ihrer Kontrollwerte festgestellt. Der Anstieg der Enzymaktivitäten im Harn war nicht von einer entsprechenden Änderung der Proteinausscheidung begleitet. Eine Auftrennung der Isoenzyme von Lactatdehydrogenase und N-Acetyl-β-*D*-glucosaminidase im Harn wies ebenfalls auf die renale Herkunft dieser Enzyme hin.

Die Veränderung der Enzymaktivitäten im Harn zeigte Art und Grad der durch Elektronenmikroskopie nachgewiesenen Nierenschädigung an.

Introduction

Normally, urine of healthy persons contains small amounts of enzymes of various origin (1), the majority originating from the kidney (1, 2). Changes in the urinary activities of these enzymes are diagnostically valuable in experimental and clinical pathology (3), because they indicate tissue damage. It is known that cellular enzymes are much more abundant in renal tubuli than in glomeruli (4), the only exception being acid phosphatase (5). Thus, tubular damage may cause an increase of renal enzyme activities in the urine.

Heavy metal ions, such as cations of mercury (6, 7, 8) and copper (9, 10) as well as the uranyl group (11, 12), are well known nephrotoxic agents with large affinity toward tubular tissue. On the other hand, the tetrathionate anion has been reported to affect the tubules and exert a highly nephrotoxic effect (13). In order to examine how the activities of urinary enzymes correlate with both localisation and intensity of renal damage, we used sodium tetrathionate. The selection of enzymes to be examined was such as to encompass cytoplasmic,

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mitochondrial, lysosomal, and membrane-bound types, and multimolecular forms of some of them.

Materials and Methods

Male Wistar rats, 150–200 g were used. The animals were distributed into two equal groups of 40, the first receiving one dose of 300 mg/kg of sodium tetrathionate (Fluka AG, Buchs SG), dissolved in 1 ml of 0.15 mol/l NaCl intraperitoneally, the second served as the control group and received only 1 ml of 0.15 mol/l NaCl by the same route. On the first, second, fourth, fifth and seventh day, groups of eight controls and eight treated animals were put into metabolic cages and their urine was collected for 24 hours. The animals were then decapitated, and kidneys excised. After washing with 0.15 mol/l NaCl the kidneys were blotted on filter paper. A sample of tissue was taken for electron microscopic examination. The remainder was freed from connective tissue, cut into small pieces, weighed, and homogenised for two minutes in 20 volumes of saline, with an ice-water cooled *Potter-Elvehjem* homogenizer at 5000 min^{-1}. After centrifugation at 12000 *g* for 20 minutes in a MSE Mistral 2 L refrigerated centrifuge, the clear supernatants were used for determination of enzymatic activities.

The volume of urine excreted during 24 h was measured, and the entire volume was centrifuged 15 min at 500 *g*. The clear supernatant was then dialysed during 3 hours against tap water at 4 °C in Visking tubes and stored at – 20 °C until analysis which was performed on the next day.

For isoenzymes determinations, urine was finally concentrated 50 times in a Minicon B 15.

All chemicals, if not otherwise mentioned, were p.a. from Boehringer, Mannheim.

The following enzymatic activities in urine and homogenates were determined: lactate dehydrogenase (EC 1.1.1.27) and glutamate dehydrogenase (EC 1.4.1.3) as described earlier (14); aspartate aminotransferase (EC 2.6.1.1) by a modification of *Wroblewski*'s method (15) and leucine aminopeptidase (EC 3.4.1.1) by a modification of *Nagel*'s method (16) using test kits manufactured by Boehringer, Mannheim; γ-glutamyltransferase (EC 2.3.2.2) by the method of *Jacobs* (17); alkaline phosphatase (EC 3.1.3.1) by the method of *Babson* et al. (18) using a test kit manufactured by Gödecke, Freiburg; β-galactosidase (EC 3.2.1.23) and N-acetyl-β-*D*-glucosaminidase (EC 3.2.1.30) fluorometrically by the methods of *Price* et al. (19) using 4-methylumbelliferyl-β-*D*-galactopyranoside and 4-methylumbelliferyl-N-acetyl-β-*D*-glucosaminide p.a. (Serva, Heidelberg) respectively as substrates.

Isoenzymes were determined after cellogel electrophoresis: lactate dehydrogenase by the method of *Thiele & Mattenheimer* (20) and N-acetyl-β-*D*-glucosaminidase by the method of *Poenaru* et al. (21).

Cellular damage was assessed with a Philips M 75 electron microscope.

Results

Enzyme activities in rat renal tissue and urine were markedly affected by treatment of the animals with sodium tetrathionate. Enzyme activities in the kidney and urine of the control group are shown on the table 1.

Over the period of observation, there was an inverse relationship between the measured activities of enzymes of cytoplasmic and mitochondrial origin in the renal tissue and in the urine. As shown in figure 1 the relative drop in renal enzymatic activity was largest with glutamate dehydrogenase which retained only a fraction of 0.30 of its initial activity on the fourth and fifth day after sodium tetrathionate administration. Lactate dehydrogenase and aspartate aminotransferase activities did not decrease as strongly, and the onset of the changes was somewhat later than those of glutamate dehydrogenase, becoming significant ($p > 0.05$) only on the fifth day following sodium tetrathionate administration. Urinary changes of the corresponding enzyme activities follow

Tab. 1. Enzyme activities in urine and kidney of the control group (N = 40).

	Urine U/24 h		Kidney U/g wet tissue	
	$\bar{x}$	SE	$\bar{x}$	SE
Lactate dehydrogenase	37.9	3.8	42.0	5.3
Glutamate dehydrogenase	13.4	1.2	1.6	0.24
Aspartate aminotransferase	30.0	2.2	21.0	2.7
N-acetyl-β-*D*-glucosaminidase	1.71	0.5	2.4	0.28
β-galactosidase	1.38	0.4	1.39	0.25
Leucine aminopeptidase	48.0	3.9	2.4	0.25
γ-glutamyltransferase	1.8	0.16	123.0	11.4
Alkaline phosphatase	72.0	13.0	74.0	18.5

$\bar{x}$ mean value
SE standard error

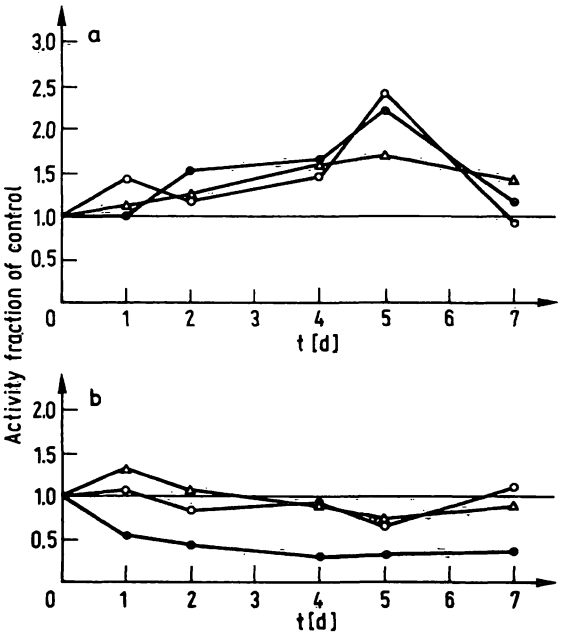

Fig. 1. Time course of the activities of lactate dehydrogenase (o—o), glutamate dehydrogenase (•—•) and aspartate aminotransferase (△—△) in urine (a) and kidney (b) in relation to controls.

exactly the opposite course, maximal enzymurias being observed on the fifth day following administration of the nephrotoxic agent, after which enzymuria tends towards normal.

Similar, but much stronger, changes were observed with urinary lysosomal glucosidase activities, which occur as early as the second day after administration of the sodium tetrathionate. These activities continue to increase sharply, and attain a maximum on the fourth day. The decrease ($p < 0.05$) of N-acetyl-β-*D*-glucosaminidase activity in renal tissue is reflected in a six-fold elevation of urinary activity (fig. 2). The activities of membrane-bound enzymes, both in renal tissue and in urine, are variably responsive to sodium tetrathionate.

There was a decrease of leucine aminopeptidase activity in the renal tissue, while alkaline phosphatase and γ-glutamyltransferase activities did not change remarkably. An elevated excretion of enzymes following treatment was observed, as in the other two groups, on the fifth day, but there was also a strong increase of alkaline phosphatase activity on the first day (fig. 3). There was no correlation between enzymurias and proteinuria. The latter was strongest on the first day following sodium tetrathionate administration, after which it decreased. On the fourth and fifth days after treatment, while the

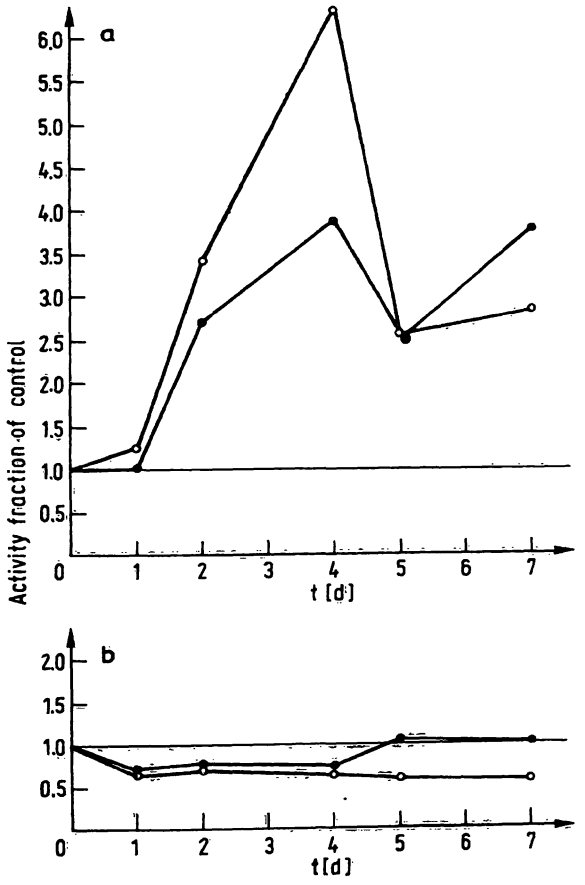

Fig. 2. Time course of the activities of N-acetyl-β-*D*-glucosaminidase (o—o) and β-galactosidase (•—•) in urine (a) and kidney (b) in relation to controls.

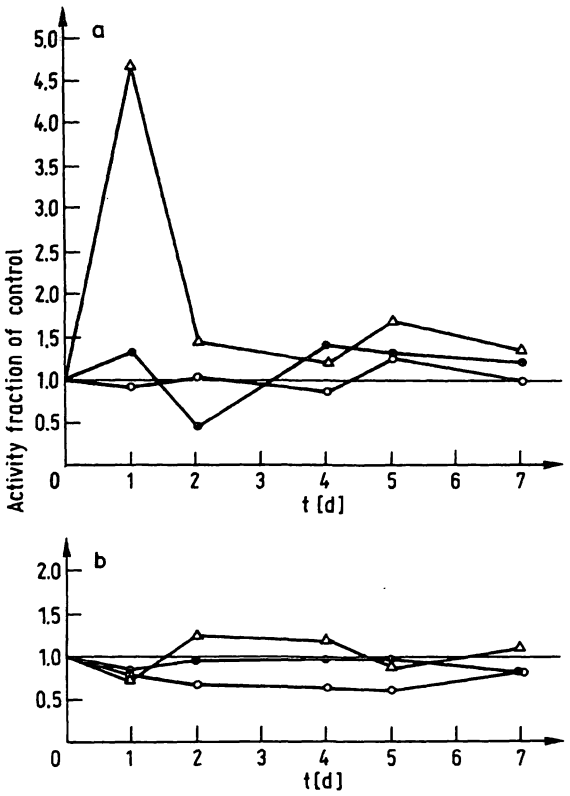

Fig. 3. Time course of the activities of leucine aminopeptidase (o—o), γ-glutamyltransferase (•—•) and alkaline phosphatase (Δ—Δ) in urine (a) and kidney (b) in relation to controls.

enzymurias were at their highest level, low proteinuria occurred in only some of the animals. This fact, together with results obtained by the differentiation of isoenzymes, suggests that the enzymes originate from the kidney (fig. 4).

Sodium tetrathionate treatment caused excretion of lactate dehydrogenase (LDH) isoenzymes LDH_1 and LDH_2, and only in a few animals LDH_5. Traces of the latter could only be detected on the first day after treatment. At the same time an increased activity of isoenzyme N-acetyl-β-*D*-glucosaminidase-B, characteristic of renal cells, was observed (fig. 5).

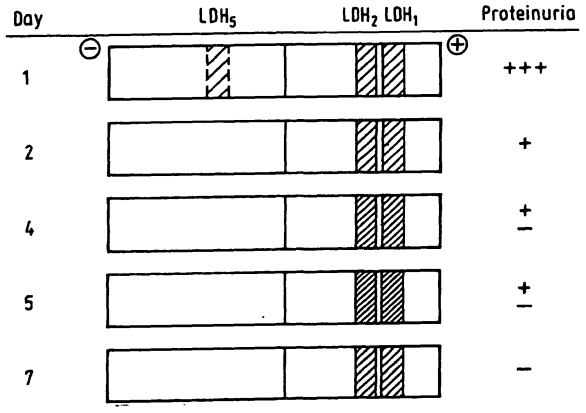

Fig. 4. The effect of sodium tetrathionate on urinary patterns of lactate dehydrogenase (LDH) isoenzymes and on the intensity of proteinuria.

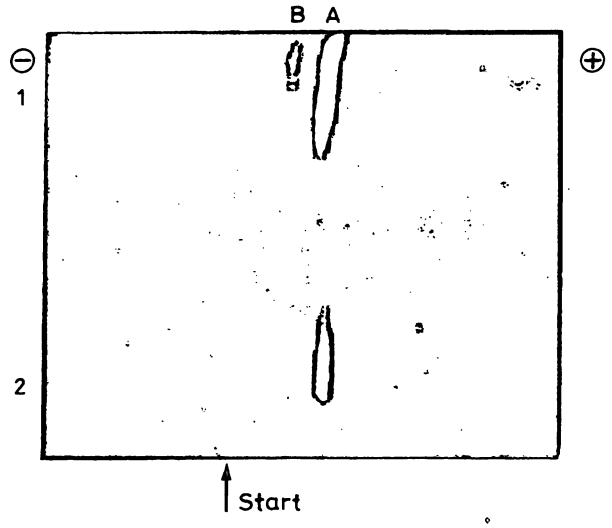

Fig. 5. Urinary patterns of N-acetyl-β-*D*-glucosaminidase isoenzymes: 1. of control, and 2. on the fourth day after administration of sodium tetrathionate.

It was noticed that the amounts of urine excreted also changed with time, and the increase of enzymurias coincided with polyuria (fig. 6).

Electron micrographs of renal tissue exhibit the following characteristics. On the first day after sodium tetrathionate treatment the cytoplasm of tubular cells is somewhat more transparent than in controls, which indicated that the cells from treated animals contain more water, which causes a swelling of mitochondria. The epithelial brush border retains its integrity at this point. On the fourth day after treatment, distinct changes were visible in the organelles. The cytoplasm had vacuolised by this time, while mitochondria had swelled considerably and many of them had cracked, and produced cytoergosomes, and there was partial destruction of epithelial brush border. On the fifth day one may observe the beginning of a regenerative process in the organelles.

The endoplasmic reticulum proliferated in lamellar form, and the numbers of mitochondria increased. The brush border reappeared by a sprouting of cellular membranes within the tubular lumen. On the seventh day, several tubular epithelial cells exhibited normal appearence, although still having some of the rejected material within their cytoplasm.

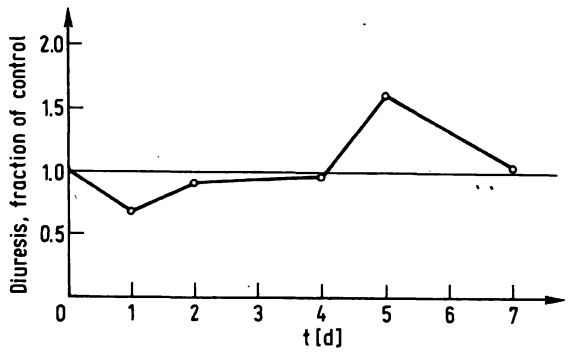

Fig. 6. Time course of diuresis in treated rats, expressed in relation to control.

Discussion

As seen from our results, the loss of the examined enzymes from renal tissue differed due to the different location of the enzymes within the nephron, as well as to different intracellular location. This behaviour was duly reflected by the corresponding activities found in the urine. All enzymes examined were those residing preponderantly in the renal tubules. Their particular locations are as follows. Lactate dehydrogenase (LDH) is entirely situated in the cytoplasm of renal cells, whereby LDH_1 and LDH_2 occur in the proximal, and some LDH_4 and LDH_5 in the distal tubules (22, 23, 24, 25). Aspartate aminotransferase is located both in cytoplasm and mitochondria of tubular cells, but a very small part occurs also in the glomeruli (26). Glutamate dehydrogenase is a specifically mitochondrial enzyme (27). β-Galactosidase and N-acetyl-β-*D*-glucosaminidase are confined to lysosomes of tubular cells (28, 29, 30) whereas γ-glutamyltransferase, leucine aminopeptidase and alkaline phosphatase are bound to the membranes and located in the epitheal cells of the brush border (31–35). The cell damaging effect of sodium tetrathionate, as observed on electron micrographs of renal tissue preparations, develops gradually. The agent crosses the brush border during the first three or four days, and enters the cells. The subsequent increase in osmolality caused obviously a considerable uptake of water into the cells and organelles, which finally disrupted their membranes. Disruption of tissue structure, with concomitant unhindered leak of cytoplasmic, mitochondrial, lysosomal, and partly membrane-bound enzymes is reflected by the gradual increase of corresponding enzyme activities in urine. Thus a small decline in renal lactate dehydrogenase appears after administration of the nephrotoxic agent, reaching a minimum on the fifth day, and thereafter tends to normalise. As a result of such changes, the urinary activity of this enzyme is already noticeable on the first day, and reaches a maximum on the fifth. The changes of renal and urinary aspartate aminotransferase show a similar pattern. It should be noted, however, that aspartate aminotransferase occurs not only in the cytoplasm but also in mitochondria. With the typical mitochondrial enzyme, glutamate dehydrogenase, a substantial decrease in renal activity starts very soon after tetrathionate administration, retaining, on the fourth and fifth day, a fraction of only about 0.30 of its initial activity. Such a pronounced loss of glutamate dehydrogenase resulted in a remarkable increase of urinary activity. These changes of mitochondrial enzymes can be explained by the pronounced damage to mitochondria observed by electron micro-

scopy. Incidentally, *Raab* (36) found a similar behaviour of urinary glutamate dehydrogenase in rats treated with sodium tetrathionate. The two lysosomal enzymes, β-galactosidase and N-acetyl-β-*D*-glucosaminidase, show similar renal changes accompanied by a marked enzymuria. The urinary activity of N-acetyl-β-*D*-glucosaminidase reaches a maximum of about sixfold elevation on the fourth day, which is in accordance with the statement that urinary activity of this enzyme is a very sensitive marker of tubular lesion (37).

As far as membrane-bound enzymes are concerned, the picture is somewhat different. While the changes of leucine aminopeptidase activities in kidney and urine show courses similar to those of the cytoplasmic enzymes, γ-glutamyltransferase behaves differently. This can be explained by the fact that leucine aminopeptidase is not so firmly bound as γ-glutamyltransferase. The changes in renal activity of γ-glutamyltransferase are only small. This enzyme, abundant in kidney, is firmly membrane-bound at the brush border, and only a very small part exists in the free form (32, 33). The small loss in tissue, and the likewise small increase in urinary γ-glutamyltransferase activity recorded on the first day is probably that of the unbound part. Along with a gradual exhaustion of renal free γ-glutamyltransferase, the activity in urine decreases. Subsequently the urinary activity increases due to dissociation of bound enzyme from the disintegrated brush border on the fourth and fifth day, as verified by electron microscopy. With alkaline phosphatase there was a large difference between relative changes in tissue and urinary activities immediately following the administration of tetrathionate; a two-phase excretion, the second phase maximum coinciding with brush border decay, was apparent. We do not offer any explanation, but we refer to others (3), who found the same kind of behaviour after renal damage caused by $HgCl_2$. Sodium tetrathionate is known to reduce the output of urine immediately after administration (3, 13). The tetrathionate caused a transient oliguria in our experiments and was followed by a substantial increase in urine output finally exceeding normal values. In addition, the urine excreted during the oliguric period contained an increased amount of protein. The increased enzymuria, occurring at the same time as polyuria, coincides with the destruction of tubular brush border. In contrast to *Sternberg* et al. (3) who examined $HgCl_2$ induced tubulonephritis in rats, we did not find any correlation between enzymuria and proteinuria. But an additional proof for the renal origin of increased enzyme activities are the patterns of urinary lactate dehydrogenase and N-acetyl-β-*D*-glucosaminidase isoenzymes mentioned above.

In summary, the detection of increased enzyme and renal isoenzymes activities in urine points to tubular damage and, therefore, may be of value in assessing the nature and intensity of renal damage.

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